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Toxicity and biological effects of three Egyptian isolates of baculovirus on the cotton leaf worm, *Spodoptera littoralis* (boisd)

Dalia M.Mahmoud[1], Ragaa K.A. Hamed[1], AlaaEdeen M. Seufi[2], Mohammad S. Salama[1], Nadia M. Lotfi Diwan[1], Akila M. El Shafei[1],
1- Department of Entomology, Faculty of Science, Ain Shams University.
2- Department of Entomology, Faculty of Science, Cairo University.

ABSTRACT

The toxicity of three different isolates of Egyptian baculovirus namely NPV_{Giza}, NPV_{Cairo} and NPV_{Alex} to S*podoptera littoralis* larvae and their effects on some biological aspects of adults were studied. The second and fourth larval instars were highly susceptible to NPV_{Cairo} than to the other isolates. The second larval instar was highly susceptible than the fourth larval instar. NPV_{Cairo} induced larval, pupal and adult malformations. It was found that the three NPV's decreased the moth longevity, fecundity, egg hatchability and altered the sex ratio.

Key Words: Bioassay- *Sopodoptera littoralis*- Baculovirus

INTRODUCTION

Human attempts at insect control have changed over time from natural to synthetic chemical control, and now again we look to natural control methods. Long-term exposure to synthetic insecticides causes many healthy problems, such as cancer, immunotoxicity and other complicated problems to man and animals. Insecticides are not specific in their action; they kill non-target and the beneficial insects, so that biological control has received more attention.

There are many biological control agents, such as bacteria, fungi and viruses (Jones, 1990 and Pawar, *et al*., 1991)**.** Baculoviridae includes nuclearpolyhedrovirus (NPV) which has polyhedron-shaped occlusion bodies. The baculovirus isolates have a limited host range, and infect only closely related species as for insects mostly of order Lepidoptera.

The main objective of the present study was to search for natural nucleopolyhedrovirus isolate(s) with better insecticidal characteristics. To accomplish this objective, the following strategy was followed: dead or diseased lepidopterous larvae were collected and viral isolation, purification and propagation were then carried out. After purification, the different viral isolates were tested for their insecticidal activities and to study their effect on malformations and some biological aspects of *Spodoptera littolaris*.

MATERIALS AND METHODS

Colony of *Spodoptera littoralis*

A laboratory colony of the Egyptian cotton leafworm, *S. littoralis* was obtained from the Department of cotton leafworm, Institute of Plant Protection, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Larvae were fed semisynthetic diet according to Shory and Hale (1965). The colony was kept

at 25 ± 2 °C, 65-70 % RH. under highly controlled conditions to avoid contamination. Such colony was used for virus propagation, bioassay test and biological observation.

Virus isolation, multiplication and purification

During 2005-2006 larvae of *S. littoralis* were collected manually from cotton and maize fields in three Governorates (Giza, Cairo and Alexandria). Larvae were examined for virus presence and the diseased ones (displaying symptoms of baculovirus infection) were immediately frozen for viral isolation.

The diseased larvae were allowed to liquefy for several days at 25 ± 2°C in plastic beakers covered with Parafilm. A crude extract was prepared by adding distilled water to each beaker and filtering the resulting mixture through a layer of naylon fabrics to remove tissue debris. The crude extract was stored at-80 °C until further processing as described by Fuxa, *et al.,* (1999).

The three isolates of *Spli*NPV were propagated by feeding third-instar larvae on semisynthetic diet, which was contaminated with virus occlusion bodies (OBs). OBs were harvested and purified from larvae cadavers as described by El Salamouny (1998).

Bioassay test

Suspension of the highly purified NPV was tested against early second and fourth larval instars. The semisynthetic diet without formalin was poured before cooling into 3 g plastic cups forming a layer of 0.5 cm. thick, which on cooling gave very smooth surface. A standard volume of 250µl of virus suspension was pipetted on to the diet surface, distributed and then left to the air. In the control treatment virus suspension was replaced by distilled water. Five different concentrations ranging from $1.8x10^7$ to $1.8x10^3$ polyhedra / ml were tested. Three replicates each of 10 larvae were used for each virus concentration. All treatments were incubated at 25°C, 60-70% RH. and natural photoperiod. Mortality was recorded at the tenth day post-infection.

Mortality data were corrected using the Abbott's formula (1925) and Lethal concentrations (LC_{25} and LC_{50}) in Polyhedral inclusion body (PIB) / ml were calculated according to the method of Litchfield and Wilcoxon (1949).

Biological observations

Early second larval instars treated with the prdetermined LC_{25} of the three NPV isolates were divided into two groups and observed till adult emergence. In the first group, longevity, and fecundity of the resultant adult females and egg hatchability were recorded. Three replicates each of 10 larvae were used for each virus. In the 2nd group, malformations and morphological abnormalities in larval, pupal and adult stages were observed.

Statistical analysis

Data were presented as mean± standard error (S.E.). The mean values were compared by t-test using the "Graphpad quick calculation" computer program (http://www.Graphpad.com). A *P*-value of ≤ 0.05 was considered indicative of statistical significance.

RESULTS AND DISCUSSION

Susceptibility of *Spodoptera littoralis* larvae to the three NPV isolates

Results obtained from bioassay tests (Table 1) demonstrated that the 2nd larval instar was more susceptible to the three tested viral isolates (NPV_{Giza}, NPV_{Cairo} and NPV_{Alex}) than the 4th larval instar. Further more, both 2nd and 4th larval instars exhibited higher susceptibility to NPV_{Cairo} than to the other isolates.

Different susceptibility levels of *S. littoralis* larvae to NPV's were observed by several authors (Klein and podoler, 1978; Komolpith and Ramakrishnan, 1975; Pawar and Ramakrishnan, 1975; Seufi and Osman 2005 and Seufi 2008). Such difference in susceptibility may be due to the difference in larval age, the number of virions contained in occlusion bodies, the method of virus administration, the feeding habit of the insect, the difference in viral strain and /or the lower number of propagation cycles of different isolates (Payne, 1982; Seufi, 2002).

Table (1): Susceptibility of *Spodoptera littoralis* larvae to three nucleopolyhedrovirus isolated from Giza, Cairo and Alex.

Virus isolates	LC_{50} (No. of PIB/ml)[#] / 95% confidence limits	
	2nd larval instar	4th larval instar
NPV_{Giza}	3.1×10^3 $6.5 \times 10^2 - 8.9 \times 10^3$	3.6×10^6 $5.6 \times 10^4 - 8.4 \times 10^6$
NPV_{Cairo}	2.2×10^3 $3.7 \times 10^2 - 6.8 \times 10^3$	2.3×10^4 $3.6 \times 10^2 - 6.5 \times 10^4$
NPV_{Alex}	1.6×10^4 $4.6 \times 10^2 - 9.5 \times 10^4$	2.7×10^5 $8.3 \times 10^4 - 9.3 \times 10^5$

[#] PIB: Polyhedral inclusion body

The results were agreeable to that of Stairs (1965), who found that the susceptibility decreased markedly as larvae of *Malacosoma disstria* grew older. In parallel, Duan and Otvos (2001) reported that mortality was higher when younger larvae of *Choristonura fumiferana* were used.

Effect of virus isolates on the biological aspects of *Spodoptera littoralis* adult female

The treatment of the 2nd larval instar with LC_{25} of the three NPV's (Table 2) decreased the moth longevity, fecundity and egg hatchability. In addition, the three NPV isolates altered sex ratio in favor of males (♂♂: total = 0.94, 0.94 and 0.77 for Giza, Cairo and Alexanria isolates, respectively) in comparison to control (♂♂: total = 0.17)

Table (2): Effect of LC_{25} of NPV_{Giza}, NPV_{Cairo} and NPV_{Alex}. isolates on the biological aspects of *Spodoptera littoralis* adult female under laboratory conditions.

Biological aspects	Tested virus (LC_{25})[#] / Mean ± S.E. [##]			Control
	NPV_{Giza} (27)	NPV_{Cairo} (18)	NPV_{Alex}. (48)	
Longevity (days)	05.00 ± 01.20[*A]	0	03.00 ± 00.90[A]	03.00 ±0 0.00
No. of egg patches/ ♀	26.00 ± 00.40[*A]	0	11.00 ± 00.40[*B]	22.00 ± 0 0.00
No. of eggs/ patch	94.95 ± 42.70[*A]	0	36.00 ± 08.60[*B]	61.00 ± 36.60
Egg hatchability %	66.00 ± 02.80[*A]	0	25.00 ± 02.30[*B]	95.00 ± 0.90

[#] No. of Polyhedral inclusion body /ml

[##] In each row, means indicated by (*) are significantly different as compared to the control and those with different letters are significantly different from each other (t-test, $P < 0.05$).

Based on the hypothesis that the efficacy of the viral biopesticide is judged by observing percent mortality and abnormalities at different applied doses (Prasad and Wadhwani, 2006). NPV_{Cairo} was more potent than other isolates, as the adult moth died after emergence. In parallel, Rothman and Myers (1996) reported that viral diseases of lepidoptera are characterized by their ability to kill infected host and to reduce the fitness of individuals that survive infection.

In consistence with our results, Duan and Otvos (2001) noticed that sublethal doses of *Choristonura fumiferana* NPV decreased longevity of adults and reduced proportions of females among survivors. However, Young (1990) reported that NPV

had little effect on pupal mortality, sex ratio and hatchability when applied to 4th larval instars of *Spodoptera ornithogalli*.

Malformation and morphological abnormalities

Two NPV isolates (NPV_{Cairo} and NPV_{Alex}) were observed for induction of malformed *S. littoralis*. Generally, the present study revealed that the resultant abnormal larvae (Figs. 1 b-f) and pupae (Figs. 2 b-f) failed to transform to the next developing stages during the normal period as in the case of control individuals. Several malformations or abnormalities were observed in treated larvae and represented by:

1- larval-pupal intermediate with larval head, thoracic legs and pupal abdomen attached with larval exuvium, (Fig. 1-b).

2- dwarfed larval pupal intermediate with larval head, thoracic legs and balloon shaped pupa from posterior end, (Fig. 1-c).

3- pupa attached with larval exuvium from posterior end, (Fig. 1-d).

4- larval-pupal intermediate with clear appearance of larvae, (Fig. 1-e).

5- dwarfed larval pupal intermediate with larval head, thoracic legs and pupal abdomen, (Fig. 1-f). Retardation in larval development was noticed in some 2nd larvae treated with NPV_{Alex} as they remained in the same instar until dying (Fig. 1 g).

6- malformed pupae that failed to transform to normal adult were represented in (Fig. 2 b-f).

7- Figure (3-b) show malformed adults with crumbled wings as compared to the normal adult (Fig. 3-a).

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Duan, L. and Otvos, I. S. (2001). Influence of larval age and virus concentration on mortality and sublethal effects of a ucleopolyhedrovirus on the Western Spruce Bud worm (Lepidoptera: Tortricidae). J. Environ. Entomol., 30: 136-146.

El Salamouny, S. (1998). Increasing efficacy of nucleopolyhedrovirus and a comparative study of an Egyptian isolate. Ph. D. Thesis, Faculty of Agriculture, Cairo University, 238 pp.

Fuxa, J.R.; Sun, J.Z.; Weidner, E.H. and LaMotte, L.R. (1999). Stressors and rearing diseases of *Trichoplusia ni*: Evidence of vertical transmission of NPV and CPV. J. Invert. Pathol., 74: 149-155.

Jones, K. A. (1990). Use of a nuclear polyhedrosis virus to control *Spodoptera littoralis* in Crete and Egypt. In Pesticide and Alternatives. J. E. Casida (ed.), Amsterdam, Elsevier., pp.131-142.

Klein, M. and Podoler, H. (1978). Studies on an application of a Nuclearpolyhedrosis virus to control population of the Egyptian cotton worm *S. littoralis*. J. Invert. Pathol., 32: 244-248.

Komolpith, U. and Ramakrishnan, N. (1975). Bioassay of the nuclear polyhedrosis virus against larval stages of *Spodoptera littoralis* and the effect of the protectants aganist ultraviolet. Proc. Indian Acad. Aci., 83:195-203.

Litchfield, J. and Wilcoxon, T. (1949). A simplified method of evaluating dose effect experiments . J. Pharmacol., 95: 99-113.

Pawar, V. M. and Ramakrishnan, N. (1975). Investigation of the nucleopolyhedrovirus of *Spodoptera litura*. III- Bioassay of virus activity. Indian J. Entomol., 37: 374-376.

Pawar, V.M.; Chandurwar, R.D.; Thombre, U.T. and Dhawandkar, S.D. (1991). Effectiveness of NPV against *Spodoptera litura* (F.) on cabbage. J. Maharashtra Agric.Univ.India., 16: 430

Payne, C. C. (1982). Insect viruses as control agents. Parasitol. 84: 35-77.

Prasad, A. and Wadhwani, Y. (2006). Pathogenic virus and insect tissue: an effective way of pest control. Current Science. 91 (6): 803-807.

Rothman, L. D. and Myers, J. H. (1996). Debilitating effects of viral diseases on host Lepidoptera. J. Invert. Pathol.67: 1-10.

Seufi, A. M. (2002). Molecular characterizatin of field isolates of entomopathogenic viruses from Egypt. Ph.D. Thesis, Dept. Entomol. Fac. Science, Cairo. Univ., 100 pp.

Seufi, A. M. (2008). Characterization of an Egyptian *S. littoralis* nucleopolhedrovirus and a possible use of a highly conserved region from polyhedrin gene for nucleopolyhedrovirus detection.Virology Journal, 5: 13.

Seufi, A. M. and Osman, G.E. (2005). Comparative susceptibility of the Egyptian cotton leafworm, *S. littoralis* to some Egyptian baculovirus isolates. Egyp. J. of biolog. pest control, 15: 21-26.

Shory, H. H. and Hale, R.L. (1965). Mass rearing of the larvae of nine noctuide species on a simple artificial medium J. Econ. Entomol., 58: 216-221.

Stairs, G. R. (1965). Quantitative differences in susceptibility of nuclear polyhedrosis virus among larval instars of the forest tent caterpillar, *Malacosoma dissteria* (Hubner). J. Invert. Pathol., 7 (4): 427-429.

Young, S.Y. (1990). Effect of nuclearpolyhedrosis virus infection in *Spodoptera ornithogalli* larvae on post larval stages and dissemination by adults. J. Invert. Pathol., 55: 69-75.

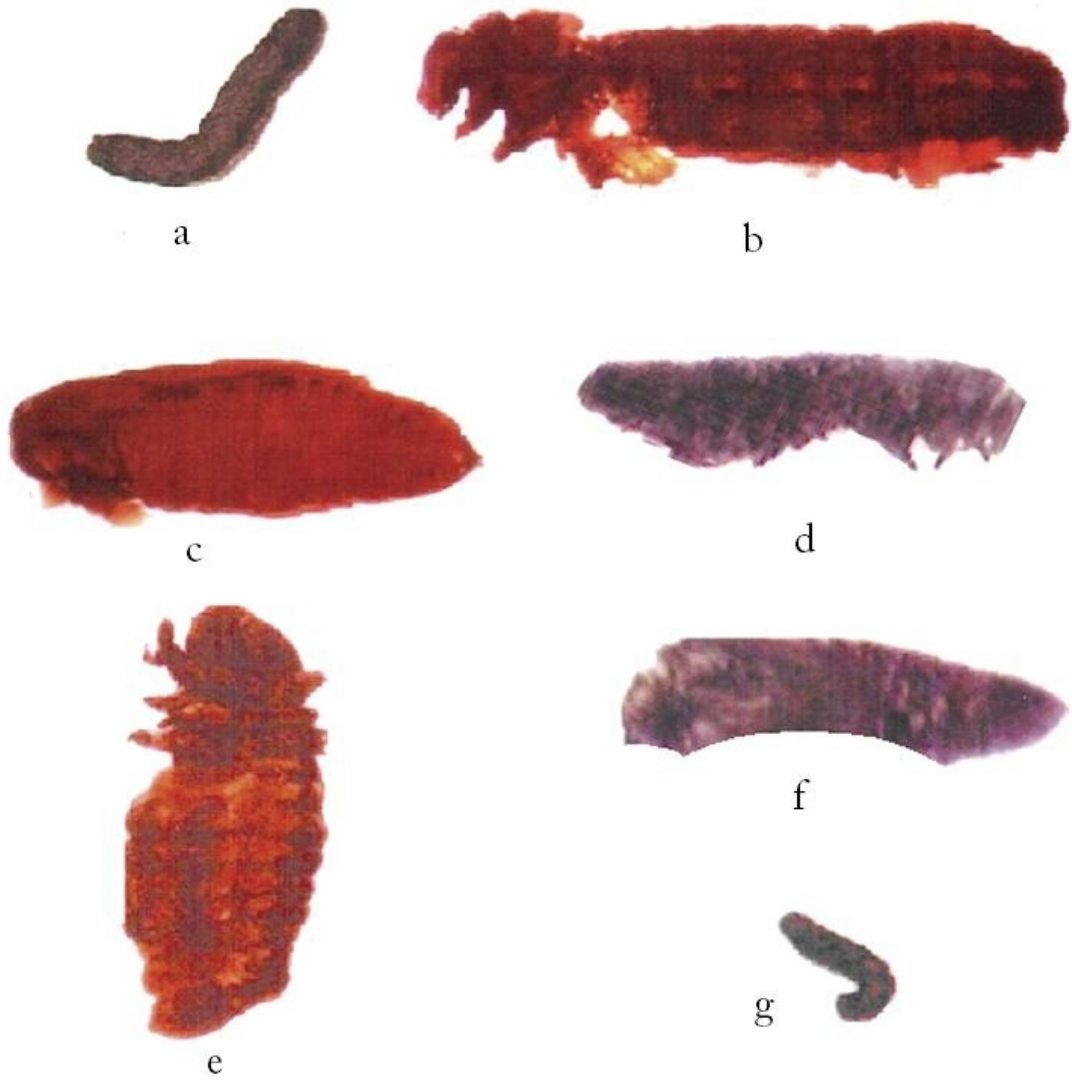

Fig. (1). Photograph of normal and malformed larvae of *Spodoptera littoralis* treated with NPV_{Cairo} and $NPV_{Alex:}$

a- Normal larva.

b- Malformed larva (NPV_{Cairo}) with larval head, thoracic legs and pupal abdomen.

c- Malformed larva (NPV_{Cairo}) showing dwarfed larva, balloon shaped pupa from posterior end.

d- Pupa (NPV_{Cairo}) attached with larval exuvium from posterior end.

e- Larval pupal intermediate (NPV_{Cairo}) with clear appearance of larva.

f- Dwarfed larval pupal intermediate (NPV_{Cairo}).

g- Malformed larva (NPV_{Alex}).

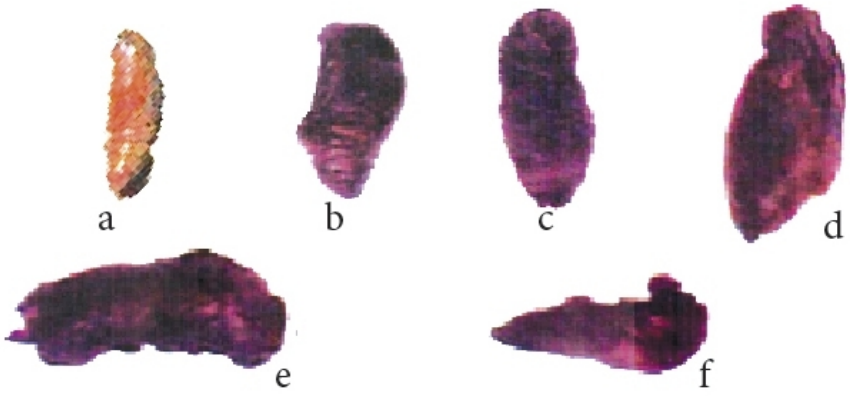

Fig. (2) Photograph of normal and malformed pupae of *Spodoptera littoralis* treated with (NPV_{Cairo}).
a - Normal pupa.
b-f - Malformed pupa failed to transform to normal adult.

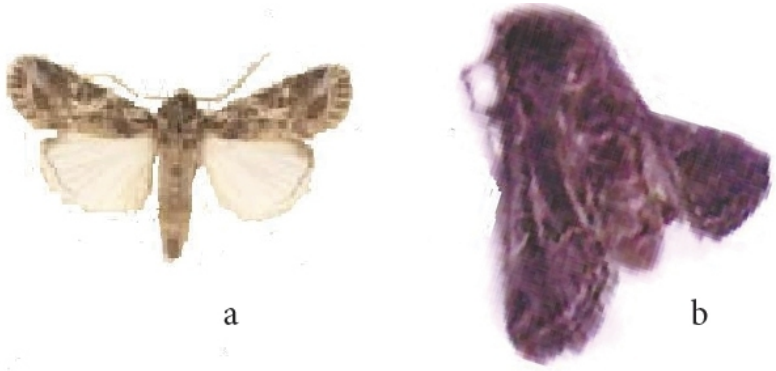

Fig. **(3)**. Photograph of normal and malformed adult of *Spodoptera littoralis* treated with (NPV_{Cairo}).
a - Normal adult.
b - Malformed adult of *S. littoralis.*

ARABIC SUMMARY

سمية والتاثيرات الحيويه لثلاث عزلات مصرية من باكولوفيروس على دودة ورق القطن سبودوبترا ليتولارس

داليا محمد محمود[1] - رجاء قلب عبد الجابر حامد[1] - علاء الدين سيوفى محمد[2] - محمد سيد سلامه[1]
ناديه محمد لطفي ديوان[1] - عقيلة محمد الشافعي[1]
1- قسم علم الحشرات- كلية العلوم -جامعة عين شمس
2- قسم علم الحشرات - كلية العلوم- جامعة القاهرة

تم فى هذا البحث تحديد سمية ثلاث عزلات مصرية من باكولوفيروس تم عزلهم من محافظات القاهرة و الجيزة و الأسكندريه و مقارنة تأثيرها على الطورين اليرقي الثاني والرابع للعائل الرئيسي وهو دودة ورق القطن المصرية.كذلك تم دراسة تأثير العزلات الثلاثة علىبعض المظاهر الحيويةللحشرات اليافعة . وأيضا تم تسجيل التشوهات التى نتجت من معاملة اليرقات بعزلتى القاهره والاسكندرية. وكانت اهم النتائج التى تم الحصول عليها هى:-

أعلى نسبة سمية كانت للفيروس الذي تم عزله من محافظة القاهرة سواء على الطور الثاني أو الرابع.

اظهر الطور الثاني لليرقات حساسية اكبر للمعاملة بالثلاث عزلات للفيروس مقارنة بالطور الرابع.

وباستخدام (LC_{25}) للثلاثة فيروسات على الطور الثاني لليرقات وجد إن هناك تأثيرا لعزلات الفيروس الثلاثة على دورة حياة الحشرة و نسبه فقس البيض ونسبة الذكور إلى الإناث وعلى خصوبة الحشرات البالغة مما أدى إلى نقص في عدد لطع البيض كما لوحظ ان عزلة القاهرة من الفيروس قد أعطت أفضل النتائج مقارنة بالعزلات الآخرى ، حيث انعدمت الخصوبة لدى الحشرات الناتجة من المعاملة بالفيروس.

وأظهرت عزلة القاهرة للفيروس تشوهات في مراحل الحشرة المختلفة من يرقه و عذراء و حشرة بالغة.

ومن نتائج هذه الدراسة اتضح انه يمكن استخدام الثلاث عزلات من باكولوفيروس و خاصة عزلة القاهرة التي تعد من افضل المبيدات الحيويةفي مكافحة دودة ورق القطن.